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Human Cytomegalovirus Latent Infection of Myeloid Cells Directs Monocyte Migration by Up-Regulating Monocyte Chemotactic Protein-1

J. Lewis Stern and Barry Slobedman

Following primary infection, human cytomegalovirus (HCMV) establishes a latent infection in hematopoietic cells from which it reactivates to cause serious disease in immunosuppressed patients such as allograft recipients. HCMV is a common cause of disease in newborns and transplant patients and has also been linked with vascular diseases such as primary and post-transplant atherosclerosis. A major factor in the pathogenesis of vascular disease is the CC chemokine MCP-1. In this study, we demonstrate that granulocyte macrophage progenitors (GMPs) latently infected with HCMV significantly increased expression of MCP-1 and that this phenotype was dependent on infection with viable virus. Inhibitors of a subset of $G_q$ proteins and PI3K inhibited the up-regulation of MCP-1 in latently infected cultures, suggesting that the mechanism underlying this phenotype involves signaling through a G-protein coupled receptor. In GMPs infected with the low passage viral strain Toledo, up-regulated MCP-1 was restricted to a subset of myeloid progenitor cells expressing CD33, HLA-DR, and CD14 but not CD1a, CD15, or CD16, and the increase in MCP-1 was sufficient to enhance migration of CD14+ monocytes to latently infected cells. Latent HCMV-mediated up-regulation of MCP-1 provides a mechanism by which HCMV may contribute to vascular disease during the latent phase of infection or facilitate dissemination of virus upon reactivation from latency.

HCMV is one of the most common infectious causes of serious disease following allogeneic organ and bone marrow transplantation (3). Following initial infection, HCMV persists in a latent state in the hematopoietic compartment and myeloid progenitor cells are a prominent site of latency. During latency, infectious virus cannot be detected and viral gene expression is significantly modified (4–8). HCMV reactivates sporadically to produce new infectious virions (9), and during immunosuppression in allograft recipients such reactivation is common and can lead to serious disease (10–12).

In recent years, evidence has emerged of a link between HCMV and restenosis, primary atherosclerosis, and posttransplant arteriosclerosis (13–16), and animal experiments (17–22) have supported the documented clinical associations (23–29). Among these findings is the observation by Streblow et al. (30) demonstrating that expression of the HCMV-encoded CC chemokine receptor, US28, in the presence of MCP-1 or RANTES, has the potential to exacerbate atherosclerosis by inducing smooth muscle cell migration. To date, no studies have specifically examined the possible contribution of latent HCMV infection to the development of vascular disease. The mechanisms driving atherosclerosis are not well understood but resemble an inflammatory reaction involving the accumulation of monocytes, T cells, and smooth muscle cells into the vessel intima (31). MCP-1 is a proinflammatory cytokine that attracts cells expressing CCR2, including monocytes, macrophages, T cells, and possibly dendritic cells (32–34). This chemokine is considered to be a major factor mediating vascular diseases such as atherosclerosis. Elevated levels of MCP-1 are observed in macrophage-rich atherosclerotic plaques and can induce infiltration of monocytes and macrophages to sites of inflammation (35, 36), smooth muscle cell proliferation, secretion of proinflammatory cytokines (37), and differentiation of monocytes into foam cells (38). In mouse models of atherosclerosis, CCR2-deficient mice exhibited reduced atherosclerosis (39) and MCP-1-deficient mice are also protected (40), further suggesting that MCP-1 contributes to the development of this disease.

Despite the importance of understanding HCMV reactivation and dissemination, it is unknown how HCMV emerges from latency to spread within the host. Reactivation results in a productive infection in permissive cell types such as macrophages and dendritic cells (DC) (4, 41). A related virus, murine CMV (MCMV) encodes a chemokine homologue MCK-2 which attracts a specific subset of myeloid cells that support MCMV infection and facilitates dissemination of the virus from initial sites of productive infection (42–45). Although HCMV encodes a putative CC chemokine (46) that may have the potential to mobilize macrophages and DC, as yet no mechanism has been demonstrated for latent HCMV to attract myeloid cells that may then support infection or facilitate dissemination.
In the current study, we report the up-regulation of MCP-1 during latent infection of granulocyte macrophage progenitors (GMPs). In addition, we show that this up-regulation enhanced chemotaxis of CD14+ monocytes to latently infected cells, suggesting that an increase in this chemokine may play a role in the spread of the virus. Furthermore, we provide evidence that this up-regulation involves PI3K. As both HCMV and MCP-1 are implicated in vascular diseases, such as atherosclerosis, these findings identify a process by which the virus may exacerbate these diseases during the latent phase of infection.

Materials and Methods

Cell and virus culture

Human fetal liver-derived GMPs were cultured and infected as previously described (47). Cell-free virus stocks were prepared by ultracentrifugation of cell culture supernatants taken from infected HFFs. Before ultracentrifugation at 112,000 × g, supernatants were clarified by centrifugation at 3360 × g for 10 min to remove cell debris. Virus titers were determined by plaque assay. For UV light inactivation of virus, stocks were exposed to UV light for 15 min at an irradiance of 4 J/m². Loss of infectivity was confirmed by plaque assay.

MCP-1 detection and FACS

Supernatant from GMP cultures were assayed using an MCP-1 ELISA kit (R&D Systems). For intracellular flow cytometry detection of MCP-1, cells were incubated with 1 μl of Golgistop (BD Biosciences) in 0.5 ml of GMP media for 12 h, washed in PBS, fixed and permeabilized using Leukoperm (Serotec) according to the manufacturer’s protocol. Staining for surface Ags was performed at 4°C before fixation and permeabilization. All mAbs and isotype Abs used for staining were mouse anti-human from BD Biosciences except anti-CD34 from Miltenyi Biotec. For experiments requiring FACS, cells were isolated based on forward scatter and side scatter properties using a FACS Diva.

Chemotaxis assays

CD14+ monocyteic cells were isolated from human PBMC with anti-CD14-paramagnetic beads from Miltenyi Biotec according to the manufacturer’s instructions. Each replicate chemotaxis assay was performed with monocytes from different donors as follows: in the bottom chamber of a 5 μm Costar permeable transwell, 25 μl of test supernatant was added to 575 μl RPMI 1640. In the top chamber 100 μl of RPMI 1640 media with 10% FCS containing 3 × 10⁵ CD14+ monocytes were added. Transwells were then incubated 2 h before counting monocytes that had migrated into the bottom chamber. For a positive control, in the bottom chamber, 4 ng of recombinant human MCP-1 (rhMCP-1) (R&D Systems) was added to 600 μl RPMI 1640; negative controls consisted of RPMI 1640 only.

PCR and RT-PCR

For DNA PCR, after washing twice in PBS, cells were suspended in lysis buffer at 5 × 10⁵ cells per 50 μl and incubated as previously described (48). Following an initial DNA denaturation step of 2 min at 94°C, PCR was performed for 40 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 2 min with primers specific for the HCMV IE1/IE2 region, IE3C, and IE1/IE2 (48) using Platinum Taq polymerase (Invitrogen). Products resulting from these PCR conditions were subjected to agarose gel electrophoresis and were also sequenced to verify their identity. For RT-PCR RNA was extracted using Ambion’s RNaqueous kit, followed by ethanol precipitation, DNase I treatment (Invitrogen), reverse transcription using random primers and Superscript III from Invitrogen, and then amplification by PCR as described above, using primers for HCMV UL83 (forward primer: GCAGAAACAGTGGAAGAGC, reverse primer: GTCTCTTCCATCGTCAGAG), HCMV glycoprotein B (gpB) (forward primer: AAGTACCCTTACCGGTG, reverse primer: GCACGTTACTCGTATTGCT), or cellular GAPDH (forward primer: CGAGATCCCTCAAAATCAA, reverse primer: GTCTCTGTTGGTGCCAGT), or cellular GAPDH (forward primer: GCAGATCCCTCAAAATCAA, reverse primer: GTCTCTGTTGGTGCCAGT). For real time PCR, DNA ystax or cDNA was amplified using Invitrogen’s Platinum SYBR Green qPCR SuperMix-UDG on a Stratagene Mx3005P with the following cycling conditions: 50°C for 2 min, 95°C for 2 min, 50 cycles of 95°C for 15 s, and 60°C for 45 s; followed by 95°C for 1 min, 55°C for 30 s, and 95°C for 30 s. The dissociation curves for all products were examined to ascertain the specificity of the amplicons. Primer sequences for MCP-1 were designed to span introns (forward primer: CCCAGCTCCTCAGGTAT, reverse primer: TGGAACTCAGGAACCCACCTTCC). Primers for HCMV DNA were IEP3C and IEP4BII (48). Primers for cellular nucleic acids used for normalization of real time PCR and real time RT-PCR results were specific for GAPDH (forward primer: ACGATCGCCGATCTTCTCTT, reverse primer: GACAAAGCCTTCCCTTCTCAG) or for β-actin (forward primer: CACAGGGAGGTGATAGCAT, reverse primer: CTCAAGTTGCGGACAA).GMPs were treated with inhibitors that target PI3Ks (2 nM, wortmannin) or G-proteins (pertussis toxin, 50 ng/ml). GMPs mock infected or infected with HCMV strain Toledo were passed every 3 days for 14 days. On day 14, 10⁵ cells were given fresh media, or fresh media containing signaling inhibitors. To assess cell viability, apoptosis and necrosis were examined by flow cytometry using Annexin V-Alexafluor-647 (Invitrogen) and propidium iodide (1 μg/ml), respectively.

Results

Latent HCMV infection up-regulates MCP-1

Our previous assessment of the host transcriptome during latent HCMV infection of primary human GMPs (47) indicated that MCP-1 transcription was up-regulated. To determine whether this increase resulted in higher secretion of this chemokine, GMP cells from 18 independent donors were either mock infected or infected with HCMV strain Toledo. Cells were passed three times per week and after 14 days media from the final passage (day 11 PI-day 14 PI) was analyzed by ELISA for MCP-1. MCP-1 levels in culture media conditioned by latently infected GMPs were higher by a factor 3.5 ± 0.6 SEM when compared with their mock-infected counterparts (Fig. 1A). This up-regulation occurred in the absence of detectable infectious virus as indicated by a lack of cytopathic effect observed on permissive HFF monolayers incubated with cell lysates or supernatants from infected GMP cultures, consistent with previous reports establishing this model of latent infection (7, 48, 49).

To ensure that this up-regulation of MCP-1 resulted specifically from infection of GMPs with HCMV, we performed two experiments. First, before infection we UV-inactivated one aliquot of viral inoculum, while leaving another aliquot untreated. Media from the final passage from mock, UV-inactivated, and infected GMP cultures were analyzed by ELISA for MCP-1. In contrast to viable virus infection, UV-inactivated virus did not increase MCP-1 expression, and levels remained comparable with those observed from mock cultures (Fig. 1B). Second, before infection we removed viros by passing the viral inocula through 0.1 μm filters, while leaving another aliquot of viral inoculum unfiltered. Although filtration removed viros, it permitted any other material of up to ∼20,000 kDa to remain. The results from three replicate experiments demonstrated that MCP-1 expression by GMPs during latent infection was not induced by 0.1 μm filtered viral inoculum (Fig. 1C), showing that removal of viros from the virus inoculum ablated the up-regulation of MCP-1. These two sets of experiments demonstrate that the increased MCP-1 expression resulted from direct infection with viable virus, and not a contaminant in the viral inoculum.

MCP-1 is upregulated in a subset of latently infected cells

GMP cultures represent a mix of myeloid progenitors at various stages of differentiation and consequently they express different levels of surface CD Ags (49). To further characterize the cells within GMP cultures with increased production of MCP-1, on day 14 PI mock and infected GMPs were analyzed by intracellular flow cytometry for MCP-1. Based on a combination of MCP-1 staining and forward and side scatter properties, only a subset of cells in GMP cultures were MCP-1+ (Fig. 2). This population was characterized by low side scatter and moderate forward scatter and is
shown in Fig. 2 as gate R1. Significantly, the proportion of cells within this subset increased 3-fold (11 to 33%) in latently infected cultures when compared with their mock infected counterparts, consistent with the 3–4-fold increase in secreted MCP-1 levels measured using ELISA.

To define the immunophenotype of the cells expressing MCP-1, we used a combination of surface and intracellular flow cytometry to examine the surface expression of myeloid cell population markers. On day 14 PI, infected GMPs stained with mAbs (or their respective isotype control Abs) were generated against cell surface markers for different subsets of monocytes (CD14, CD16), granulocytes (CD15), myeloid progenitor cells (CD33), hematopoietic progenitor cells (CD34), DC (CD1a), or MHC class II (HLA-DR). This was followed by fixation and permeabilization and staining for MCP-1. Although there was some variability in the total proportion of MCP-1 expressing cells in GMP cultures when stained with different combinations of Abs due to altered data acquisition settings, this analysis identified the population of cells expressing MCP-1 as being CD33\(^+\)/HLA-DR\(^-\) with varying levels of expression of CD14 but not expression of CD16, CD1a or CD34 (Fig. 3 and data not shown). Although some of the cells within the culture were CD15 positive, <3% of cells expressing MCP-1 also expressed CD15 (Fig. 3). Based upon this cell surface marker analysis, the MCP-1-expressing cells in our GMP cultures most closely resemble that of monocytes and their differentiating progenitors.

GMPs are highly susceptible to latent HCMV infection, frequently yielding rates of infection >90% at ~10 genome copies per cell (7). However, because only a subset of cells increased MCP-1 expression, we considered the possibility that in our experiments only a subset of cells were infected. To determine the proportion of cells in our cultures infected with HCMV, on day 14 PI, the populations of MCP-1 positive and MCP-1 negative GMPs...
were separated by FACS from three independent replicate GMP cultures, and GMP cell lysates from 5 × 10^4 cells were analyzed for viral genomic DNA by PCR. Using primers IEP3C and IEP4BII specific for the HCMV IE1/IE2 genomic region (48), a positive PCR signal at the predicted size (387 bp) was detected in both the MCP-1 positive and MCP-1 negative populations from all three GMP cultures, and in a positive control consisting of lysate from productively infected HFFs (Fig. 4A). Viral DNA was not detected in mock infected GMPs, nor in samples in which template DNA was omitted. To determine whether MCP-1 positive and MCP-1 negative GMPs contained different levels of infection, the relative amount of viral DNA in each population was assessed by real-time PCR on lysates from each population from each of 3 replicate GMP cultures. Following normalization to cellular nucleic acid, the relative HCMV DNA level was determined (Fig. 4B). This analysis revealed an ~2-fold greater amount of viral DNA in MCP-1 negative cells in comparison to MCP-1 positive cells, although this difference was not statistically significant. It was therefore concluded that both MCP-1 positive and MCP-1 negative GMPs from infected cultures harbored the viral genome, and that the levels of infection in each population were similar. These data show that while the viral genome was maintained in all GMPs, only a subset of these cells expressed and up-regulated MCP-1. To further confirm that the viral genome was maintained in these cells in the absence of viral productive infection, RNA extracted from each sorted GMP population was subjected to RT-PCR with primers specific for transcription from HCMV UL83 and UL55 (gB coding gene). These were chosen as markers of potential productive infection as UL83 and gB transcripts are expressed during the productive phase of infection with early and late kinetics, respectively, but have not previously been reported to be expressed in latently infected myeloid progenitor cells (8, 50, 51). UL83 and gB transcripts were readily detected in RNA extracted from productively infected HFFs but were not detectable in infected GMP RNA from either MCP-1 positive or MCP-1 negative populations from any of three independent replicate experiments (Fig. 4C). Cellular GAPDH was detected in all samples, confirming the integrity of the RNA used for these analyses. Taken together with the lack of detectable infectious virus and the presence of viral DNA, these results provide additional evidence that the
observed up-regulation of MCP-1 by HCMV in GMPs was a consequence of viral latency.

**Induction of MCP-1 expression in GMPs relies on G-proteins and phosphatidylinositol-3-kinase**

The induction of MCP-1 expression can occur in response to a range of stimuli that can initiate signaling and transcription through different cascades. In a number of contexts, PI3Ks mediate intracellular signaling cascades that result in MCP-1 induction, including mast cells, neutrophils, monocytes, and macrophages (52–58). PI3Ks are structurally categorized into three classes (59–61). Class I PI3Ks, subdivided into classes IA and IB, mediate signals through receptor tyrosine kinases (RTK) and G-protein coupled receptors (GPCR) respectively. To assess any contribution that PI3Ks or GPCRs may have played in the induction of MCP-1 by latent HCMV, mock infected or HCMV strain Toledo infected GMPs on day 14 PI were treated with pertussis toxin, a commonly used inhibitor of a broad range of Gα-proteins, or wortmannin a pan-PI3K inhibitor. After 16 h, cells were harvested and MCP-1 transcription was analyzed by quantitative RT-PCR. When compared with levels of mRNA for MCP-1 in untreated cells, levels of MCP-1 transcription were not significantly altered in mock infected cultures when treated with either wortmannin or pertussis toxin (Fig. 5). In contrast, levels of MCP-1 transcription in infected GMPs was substantially reduced by inhibitors for both PI3K and G-proteins (Fig. 5). Assessment of apoptosis and necrosis by flow cytometry using Annexin V and propidium iodide staining, respectively, confirmed that these changes in MCP-1 expression were not a result of nonspecific effects of the inhibitors on cell viability (data not shown). These data suggest that the up-regulation of MCP-1 transcription in infected GMPs involves a GPCR and opens the possibility that signals from a GPCR may be mediated by class IB PI3K. In addition, it suggests that the pathway(s) regulating MCP-1 expression in infected GMPs differs from that used normally in uninfected GMPs.

**Latent HCMV enhances chemotaxis of CD14+ monocytes via MCP-1**

MCP-1 acts to attract cells such as monocytes. To determine whether latent HCMV infection directed chemotaxis of CD14+ monocytes, we tested the ability of GMP cells to attract CD14+ monocytes. On day 14 PI mock and infected GMPs were transferred at equal densities to complete RPMI 1640 media for 48 h at which time supernatants were used in a chemotaxis assay with CD14+ monocytes. Positive and negative control treatments consisting of media with or without the addition of 4 ng of rhMCP-1 were included. The mean number of migrating monocytes in the bottom chamber of transwells for each treatment was determined by counting multiple fields of view under 200× magnification. In response to media alone, only basal levels of migration were observed, whereas migration was rapidly induced in the presence of rhMCP-1, confirming that these cells migrated specifically in response to MCP-1 (Fig. 6A). In a comparison of chemotaxis of monocytes (from three different donors) to mock vs infected GMP cells (also from three different donors), a significant increase in migration of monocytes to infected cell supernatants was observed. This indicated that latent infection of GMPs induced the secretion of a chemotactic signal (Fig. 6A).

To determine whether the increased chemotaxis of monocytes resulted from MCP-1, we used a neutralizing Ab against MCP-1 to specifically block chemotaxis to this chemokine. Cells from the MCP-1+ population from both mock and infected cultures were isolated by FACS and cultured at equal densities for 48 h before being used in a chemotaxis assay. Each treatment was performed either in the presence or absence of 7.5 μg of a neutralizing Ab.
against MCP-1. As a methodological control, chemotaxis of monocytes to an unrelated chemotactic stimulus, the bacterial peptide FMLP was included, both in the presence and absence of 7.5 μg of neutralizing Ab to MCP-1. Migration of monocytes to rhMCP-1 was almost completely blocked by the addition of neutralizing Ab against this chemokine, confirming the ability of this Ab to neutralize the chemotactic properties of this chemokine (Fig. 6B). In contrast, migration of monocytes to FMLP was unaffected by the presence of anti-MCP-1 neutralizing Ab indicating that these cells retained the capacity to migrate in the presence of this neutralizing Ab (Fig. 6B). However, chemotaxis of monocytes to supernatants from both infected and mock infected GMP cultures was almost completely inhibited by the presence of anti-MCP-1 neutralizing Ab (Fig. 6B) indicating that the ability of these supernatants to attract CD14+ monocytes resulted almost exclusively from the presence of MCP-1 secreted by these cells. In a further two independent replicate experiments, migration to infected supernatants was also blocked to similar levels by anti-MCP-1 (data not shown). These results demonstrate that the subpopulation of latently infected GMPs shown to up-regulate expression of MCP-1 induced greater chemotaxis than mock-infected cells and that this increased chemotaxis was a consequence of the expression of MCP-1 and not another chemokine.

Discussion

Despite the importance of latency to the success of HCMV as a human pathogen, this phase of infection remains very poorly understood. In particular, very little is known about the impact of latent infection on the host. In this study, we report that latent infection of primary human GMPs resulted in higher secretion of MCP-1 compared with mock-infected cells. It was important to demonstrate that this up-regulation was due specifically to infection with viable virus, as it has previously been reported that an activity found in the medium of unpurified virus stock was sufficient to transiently induce MCP-1 expression in HFFs (62). In our study, virus subjected to UV inactivation or viral inocula filtered of virions lost the ability to up-regulate MCP-1 in GMPs, demonstrating that this up-regulation required infection with viable virus. Our finding that MCP-1 is up-regulated during latency contrasts with the down-regulation of MCP-1 mRNA observed in HFFs productively infected with purified HCMV (62), highlighting a fundamental difference in the MCP-1 response by different cell types during latent and productive phases of infection. Significantly, the up-regulated secretion of MCP-1 during latent infection was sufficient to significantly increase the migration of monocytes to latently infected cells. There are two main implications of these findings. First, as both HCMV and MCP-1 are implicated in vascular diseases such as atherosclerosis, the up-regulation of MCP-1 during latent infection identifies a mechanism by which the virus may accelerate the development of vascular disease during this phase of infection. Second, there is a significant overlap between the types of cells attracted by MCP-1 and the types of cells that are known to support HCMV infection, suggesting that an increase in this chemokine may help to facilitate spread of the virus. This may be particularly advantageous during the initial stages of reactivation from latency.

It is possible that naive monocytes or other cells may be recruited as targets of infection to sites of latency by latently infected GMPs. Alternatively, latently infected GMPs may continue to up-regulate MCP-1 as they mature into monocytes and then macrophages, from which virus may reactivate and spread to MCP-1-responsive leukocytes. Although the effects of latent infection of GMPs on the MCP-1 response and its functional outcome had not been previously examined, studies of nonpermissive naive monocytes exposed to HCMV have provided additional insights into the effects of HCMV on cells of the myeloid lineage. It has been reported that HCMV inhibits cytokine-induced maturation of macrophages from monocytes following exposure to virus (63), although other studies have provided evidence that HCMV induces monocyte to macrophage differentiation (64, 65). Indeed, Yurochko and colleagues have proposed a model of HCMV dissemination, by which monocytes, which at the time of infection are nonpermissive to viral replication, are induced to differentiate into macrophages which then become permissive for replication of the original input virus (64, 65). Although it remains to be determined whether such a mechanism of dissemination would interface directly with latently infected GMPs, our finding that MCP-1 producing GMPs have features of monocytic cells raises the possibility that pre-existing latent infection in GMPs may act as the precursors for a monococyte-to-macrophage-based model of virus dissemination.

The proposed mechanisms by which HCMV may exacerbate vasculopathies are several but include smooth muscle cell proliferation and/or migration (30) and induction of C-reactive proteins (66). Up-regulated MCP-1 in latently infected monocytes infiltrating sites of vascular inflammation may exacerbate inflammation and other features of these vascular diseases. Moreover, Streblow et al. (30) showed that smooth muscle cells infected with HCMV in vitro undergo migration in response to MCP-1, a process important to the thickening of arterial intima that exacerbates atherosclerotic plaques. This migration was dependent on MCP-1 or RANTES binding to the HCMV-encoded CC chemokine receptor, US28. Thus, in addition to functioning directly to recruit cells to sites of vascular damage, latently infected cells entering vascular sites already harboring HCMV and expressing US28 may exacerbate the existing condition by MCP-1/US28 interactions. Taken together with the pivotal role of MCP-1 in primary atherosclerosis and posttransplant arteriosclerosis, this elevated expression of MCP-1 by latently infected myeloid progenitors strongly warrants further investigation of latently infected myeloid progenitors in vivo. However, the very low levels of natural latent infection and the difficulty in obtaining sufficient numbers of latently infected cells remains a major complicating factor (7, 67). Consequently, experimental models of latent infection of primary hematopoietic cell types will continue to play a major role in guiding studies of HCMV latency and its impact on the host.

In addition to showing an increased production of MCP-1 by infected GMPs, using intracellular flow cytometry, a subset of latently infected cells responsible for the increase was identified. These cells were CD33+/HLA-DR+/CD14+/− but they did not express significant levels of markers for DC (CD1a), granulocytes (CD15), or the low affinity IgG receptor (CD16), which may define a population of residential, noninflammatory monocytes (68). This phenotype in our GMP cultures is very similar to the surface expression observed in monocytes and their committed progenitors in vivo suggesting that the identity of the cell type expressing MCP-1 in our cultures most resembles such cells.

The ability of the virus to disseminate from sites of latency may be mediated by the recruitment of cells that support HCMV infection and replication, such as macrophages or DC, both of which have been reported to express CCR2 (32, 33, 69, 70). In this study, we show that latently infected MCP-1+ cells were able to attract greater numbers of CD14+ monocytes than mock-infected cells. CD14+ monocytes and macrophages or DC resulting from differentiation have been shown to support HCMV infection (4, 41, 64, 65, 71–73). Thus, attracting these cells may enable the virus to spread more efficiently following reactivation from latency.
Interestingly, a role for CC chemokine expression in virus dissemination has been described for the MCMV-encoded chemokine homologue MCK-2, which causes a marked increase in myelomonocytic cell recruitment to initial sites of infection with a consequence of enhanced dissemination of virus (42–45).

MCP-1 is expressed in response to inflammatory mediators (e.g., LPS, IL-1β, TNF-α, IFN-α/β, IFN-γ, IL-6, IL-4, IL-10), but the effect of these can be context dependent (74–79). We recently reported that latent infection of GMPs also significantly up-regulates transcription of S100B (47), a secreted protein that may induce MCP-1 expression (80). However, addition of exogenous human S100B homodimer (at equivalent levels to those measured by S100B ELISA in infected GMP supernatants did not up-regulate MCP-1 protein levels in uninfected GMPs or enhance the up-regulation of MCP-1 by infected GMPs (data not shown), suggesting that exogenous S100B was not responsible for generating this phenotype in this cell type. Similarly, although human IL-10 has been reported to induce MCP-1 in monocytes (79, 81) and HCMV encodes homologues of human IL-10 during both productive and latent phases of infection (82–84), we were not able to demonstrate an up-regulation of MCP-1 protein secretion by GMPs treated with exogenous rhIL-10, or the virally encoded homologues cmvIL-10 or LAcmvIL-10 (data not shown).

To gain an insight into the mechanism of MCP-1 up-regulation during latent infection, we used two inhibitors of common signal-regulating homologues of human IL-10 during both productive and latent infection. We recently reported that latent infection of GMPs also significantly up-regulates transcription of S100B (47), a secreted protein that may induce MCP-1 expression (80). However, addition of exogenous human S100B homodimer (at equivalent levels to those measured by S100B ELISA in infected GMP supernatants did not up-regulate MCP-1 protein levels in uninfected GMPs or enhance the up-regulation of MCP-1 by infected GMPs (data not shown), suggesting that exogenous S100B was not responsible for generating this phenotype in this cell type. Similarly, although human IL-10 has been reported to induce MCP-1 in monocytes (79, 81) and HCMV encodes homologues of human IL-10 during both productive and latent phases of infection (82–84), we were not able to demonstrate an up-regulation of MCP-1 protein secretion by GMPs treated with exogenous rhIL-10, or the virally encoded homologues cmvIL-10 or LAcmvIL-10 (data not shown).

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